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PATENT EXTENSION
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U. S. Patent No. 5,162,504

Issued: November 10, 1992

Inventor: Julius S. Horoszewicz

Title: MONOCLONAL ANTIBODIES TO A NEW ANTIGENIC MARKER IN EPITHELIAL PROSTATIC CELLS AND SERUM OF PROSTATIC CANCER PATIENTS

Certificate Under 37 C.F.R. 1.8(a)

I hereby certify that the correspondence is being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on

Paid 5-6-96

December 20, 1996
Date of deposit

W. Scott McNees
W. Scott McNees

Reg No. 33,964
Reg. No.

Application for Extension of Patent Term Under 35 U.S.C. 156

Honorable Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicants hereby request extension of the term of U.S. Patent No. 5,162,504, pursuant to the provisions 35 U.S.C. 156 and 37 C.F.R. 1.710, et seq. U.S. Patent 5,162,504, assigned to the applicant Cytogen Corporation, contains two claims covering a monoclonal antibody 7E11-C5 and the hybridoma which produces the monoclonal antibody. The monoclonal antibody 7E11-C5 covered by Claim 1 of the patent is a component of a product as defined in 37 C.F.R. 1.710,

known as ProstaScint™, which has been subjected to regulatory review in the FDA, and has recently been approved for marketing.

In compliance with 37 C.F.R. 1.740, applicants provide the following:

1) Complete identification of the approved product:

The product will be sold under the trade name ProstaScint™, and is generally called Capromab Pendetide. The product is a murine monoclonal antibody, 7E11-C5.3, conjugated to a linker-chelator, glycyl-tyrosyl-(N,ϵ-diethylenetriaminepentaacetic acid)-lysine hydrochloride (GYK-DTPA-HCl). The antibody is of the IgG1 kappa subclass, and is directed against a glycoprotein expressed by prostate epithelium known as Prostate Specific Membrane Antigen (PSMA). A product insert is attached hereto as Exhibit 1.

2) The regulatory review of the product occurred under the Federal Food, Drug and Cosmetic Act, and 21 C.F.R. 300, et seq.

3) The product received permission for commercial marketing from the FDA on October 28, 1996. A copy of the approval letter from FDA is attached as Exhibit 2.

4) The product is not a drug product, but is a biologic product used for in vivo imaging. The biologically active ingredient of the product is the monoclonal antibody 7E11-C5.3. This monoclonal antibody has not been previously approved for commercial marketing or use under the Federal Food, Drug, and Cosmetic Act, the Public Health Service Act, or the Virus-Serum, Toxin Act. The linker-chelator portion of the product was previously approved for commercial use on December 29, 1992 by the FDA, under the Federal Food, Drug, and Cosmetic Act and Title 21, Code of Federal Regulations, for use in combination with a different antibody for in vivo imaging of colorectal and ovarian cancer.

5) The present application for extension of the patent term is being submitted within the sixty day period permitted pursuant to 37 C.F.R. 1.720 (f). The last day on which this application could be submitted is December 26, 1996.

6) Applicants are requesting an extension of the term of U.S. Patent No. 5,162,504, in the name of Julius S. Horoszewicz, issued on November 10, 1992 and which is presently due to expire on November 10, 2009.

7) A copy of U.S. Patent No. 5,162,504 is attached hereto as Exhibit 3.

8) A copy of a Maintenance Fee Statement, indicating that the first maintenance fee has been paid, is attached as Exhibit 4.

9) U.S. Patent No. 5,162,504 claims the approved product. Specifically, claim 1 of the

patent is directed to a monoclonal antibody produced by hybridoma cell line 7E11-C5, ATCC Designation HB 10494. The monoclonal antibody binds specifically to an epitope present on a membrane associated antigen of human prostatic cancer epithelium, and normal prostatic epithelium, and does not bind to non-prostatic antigens present in other tissues. The approved product comprises the monoclonal antibody covered by claim 1, conjugated to a linker-chelator (see Exhibit 1).

10) The investigational new drug (IND) application was received by the FDA on September 26, 1989 and became effective thirty days later on October 26, 1989, under IND #3311. A letter dated October 14, 1989 confirming the IND submission is attached as Exhibit 5.

The Product License Application (PLA) was submitted January 12, 1995 and was assigned Reference No. 95-0041. The PLA was approved on October 28, 1996.

11) Significant marketing activities, and relevant dates during the regulatory review period:

In the second half of 1995, a training program was initiated to train radiologists and nuclear medicine physicians in the proper use of the product. By September 1996, forty medical centers had received the training and became qualified to use the product. In August 1996, applicant entered into an exclusive comarketing agreement with a major medical company for the marketing and sales of the product. In October 1996, a final Marketing Plan was completed.

12) In the opinion of the applicant, U.S. Patent No. 5,162,504 is eligible for the extension applied for herein. The length of the extension of the term requested is the period of time from November 10, 2009 through October 28, 2010. This period was calculated as follows:

Under 37 C.F.R. 1.775 (c) the regulatory review period is the period from October 26,1989 to October 28,1996, i.e. 2557 days.

Under 37 C.F.R. 1.775 (d) (1), the number of days during the review period prior to the patent issue date, i.e. 1011 days, were subtracted, to leave 1546 days. A further 946 days were subtracted under §1.775 (d) (1) (iii), to leave 600 days. Adding 600 days to the expiration date gives a date of July 3, 2011.

Under §1.775 (d) (3), adding 14 years to the date of product approval, i.e. October 28, 1996, yields a date of October 28, 2010.

Under §1.775 (d) (4), the earlier of the dates under (d) (2) and (d) (3) is October 28, 2010.

Under §1.775 (d) (5), adding 5 years to November 10, 2009 yields November 10, 2014; selecting the earlier of this date and the date under (d) (4) yields October 28, 2010, which is the date to which the subject patent is entitled to be extended.

13) Applicant acknowledges a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension requested.

14) A check in the amount of $1,030.00 to cover the fee for this application is enclosed.

15) Inquiries and correspondence relating to this application may be directed to:

W. Scott McNees
Cytogen Corporation
600 College Road East
Princeton, NJ 08540
Phone: (609) 520-3062
Fax: (609) 951-9298

16) Applicant's undersigned attorney certifies that this application is being submitted in duplicate.

DECLARATION UNDER 37 C.F.R.1.740 (b)

The undersigned attorney for applicant declares as follows:

1. I am a patent attorney authorized to practice before the U. S. Patent and Trademark Office, and have general authority from the owner of U. S. Patent No. 5,162,504, namely, Cytogen Corporation, to act on behalf of said owner in patent matters.

2. I have reviewed and understand the contents of the attached application under 37 C.F.R. 1.740 for extension of U. S. Patent No. 5,162,504.

3. I believe that U. S. Patent No. 5,162,504 is subject to extension pursuant to 37 C.F.R. 1.710.

4. I believe an extension of the length claimed, i.e. until October 28, 2010, and is justified under 35 U.S.C. 156 and the applicable regulations.

5. I believe that U. S. Patent No. 5,162,504 meets the conditions for extension of the term of a patent as set forth in 37 C.F.R. 1.720.

W. Scott McNees

W. Scott McNees
Reg. No. 33,964
Attorney for Applicant

Best Available Copy

Exhibit 1

ProstaScint™ Kit (Capromab Pendetide)

Kit for the Preparation of Indium In 111 Capromab Pendetide For Intravenous Use Only

DESCRIPTION

ProstaScint™ (Capromab Pendetide) is the murine monoclonal antibody, 7E11-C5.3, conjugated to the linker-chelator, glycyl-tyrosyl-(N, ε-diethylenetriaminepentaacetic acid)-lysine hydrochloride (GYK-DTPA-HCl). The 7E11-C5.3 antibody is of the IgG1, kappa subclass (IgG1κ). This antibody is directed against a glycoprotein expressed by prostate epithelium known as Prostate Specific Membrane Antigen (PSMA). The PSMA epitope recognized by monoclonal antibody (MAb) 7E11-C5.3 is located in the cytoplasmic domain. Expression of this glycoprotein has not been demonstrated on any other adenocarcinomas or transitional cell cancers tested. The antibody is produced by serum-free *in vitro* cultivation of cells, and purified by sequential protein isolation and chromatographic separation procedures.

Each ProstaScint™ kit consists of two vials which contain all of the non-radioactive ingredients necessary to produce a single unit dose of Indium In 111 ProstaScint™, an immunoscintigraphic agent for administration by intravenous injection only. The ProstaScint™ Vial contains 0.5 mg of capromab pendetide in 1 mL of sodium phosphate buffered saline solution adjusted to pH 6; a sterile, pyrogen-free, clear, colorless solution that may contain some translucent particles. The vial of sodium acetate buffer contains 82 mg of sodium acetate in 2 mL of Water for Injection adjusted to pH 5-7 with glacial acetic acid; it is a sterile, pyrogen-free, clear, and colorless solution. Neither solution contains a preservative. Each kit also includes one sterile 0.22 μm Millex® GV filter, prescribing information, and two identification labels.

The sodium acetate solution must be added to the sterile, non-pyrogenic high purity Indium In 111 Chloride solution to buffer it prior to radiolabeling ProstaScint™. The immunoscintigraphic agent Indium In 111 Capromab Pendetide (Indium In 111 ProstaScint™) is formed after radiolabeling with Indium In 111.

Physical Characteristics of Indium In 111

Indium In 111 decays by electron capture with a physical half-life of 67.2 hours (2.8 days). The energies of the photons that are useful for detection and imaging studies are listed in TABLE 1.

TABLE 1 - Indium In 111 PRINCIPAL RADIATION EMISSION DATA[1]

Radiation	Mean % per Disintegration	Mean Energy (keV)
Gamma 2	90.2	171.3
Gamma 3	94	245.4

External Radiation

The exposure rate constant for 37 MBq (1 mCi) of Indium In 111 is 8.3×10^{-5} C/kg/hr (3.21 R/hr). The first half-value thickness of lead (Pb) for Indium In 111 is 0.023 cm. A range of values for the relative attenuation of the radiation emitted by this radionuclide that results from the interposition of various thicknesses of Pb is shown in TABLE 2. For example, the use of 0.834 cm of lead will decrease the external radiation exposure by a factor of about 1,000.

TABLE 2 - Indium In 111 RADIATION ATTENUATION OF LEAD SHIELDING[2]

Shield Thickness (Pb) cm	Attenuation Factor
0.023	0.5
0.203	10^{-1}
0.513	10^{-2}
0.834	10^{-3}
1.120	10^{-4}

These estimates of attenuation do not take into consideration the presence of longer-lived contaminants with higher energy photons, namely Indium In 114m/114.

To allow correction for physical decay of Indium In 111, the fractions that remain at selected intervals before and after the time of calibration are shown in TABLE 3.

TABLE 3 - Indium In 111 PHYSICAL DECAY CHART, HALF-LIFE 67.2 HOURS (2.8 DAYS)

Hours	Fraction Remaining
-48	1.64
-36	1.44
-24	1.28
-12	1.13
0*	1.00
12	0.88
24	0.78
36	0.69
48	0.61
60	0.54
72	0.48
84	0.42
96	0.37
108	0.33
120	0.29
132	0.26
144	0.23

* Calibration Time

CLINICAL PHARMACOLOGY

Pharmacodynamics

Prostate Specific Membrane Antigen is expressed in many primary and metastatic prostate cancer lesions, and *in vitro* immunohistologic studies have shown 7E11-C5.3 to be reactive with over 95% of the prostate adenocarcinomas evaluated. In general, PSMA expression by prostate cancer cells is either unchanged or increased in patients treated with hormonal therapy (see PRECAUTIONS, Drug Interactions). The 7E11-C5.3 antibody is immunoreactive with normal and hyperprophic adult prostate tissue. In clinical studies of patients with prostate cancer, Indium In 111 ProstaScint™ (Capromab Pendetide) localized to the prostate, and some known primary and metastatic tumor sites.

Non-antigen-dependent localization, suspected to be secondary to catabolism, has been observed in the liver, spleen, and bone marrow. Although there is variation among individuals, there may also be localization and imaging activity in the bowel, blood pool, kidneys, urinary bladder, and genitalia. Intracellular localization of 7E11-C5.3 has been observed in histochemically prepared tissue sections from normal adult skeletal and cardiac muscle, although primate studies revealed no specific localization to these tissues.

Pharmacokinetics

Based on data obtained from clinical studies, Indium In 111 ProstaScint™ demonstrated a monoexponential elimination pattern with a terminal-phase half life of 67 ± 11 hours (mean± SD). Approximately 10% of the administered radioisotope dose is excreted in the urine during the 72 hours following intravenous infusion. The pharmacokinetics of Indium In 111 ProstaScint™ are characterized by slow serum clearance rate (42 ± 22 mL/hr) and small volume of distribution (4 ± 2.1 L).

CLINICAL STUDIES

Indium In 111 ProstaScint™ (Capromab Pendetide) has been administered in single doses to over 600 patients in clinical studies, and in repeat administrations (2 to 4 infusions) to 61 patients. A 0.5 mg dose was determined to be the lowest effective dose. The imaging performance of Indium In 111 ProstaScint™ was evaluated in a phase 2 and a phase 3 trial in each of two clinical settings: (1) patients with clinically-localized prostate cancer who were at high risk for metastases and (2) patients with a high clinical suspicion for occult recurrent or residual prostate cancer.

Imaging Performance in Newly-Diagnosed Patients

In one of two open label, multi-center, uncontrolled pivotal phase 3 trials, 160 patients with a tissue diagnosis of prostate cancer who were considered at high risk for lymph node metastases underwent Indium In 111 ProstaScint™ immunoscintigraphy prior to scheduled staging pelvic lymphadenectomy. High risk was defined as at least one of the following: (1) prostate specific antigen (PSA) ≥10x the upper limit of normal & Gleason score ≥7; (2) prostatic acid phosphatase above the upper limit of normal; (3) equivocal evidence of lymph node metastases on CT or ultrasound & PSA ≥8x the upper limit of normal; (4) Gleason score ≥8; or (5) clinical stage C & Gleason score ≥6. All patients had been evaluated for metastatic disease using standard non-invasive imaging techniques, and were considered to have clinically-localized prostate cancer. The Indium In 111 ProstaScint™ images were interpreted on-site, and the reader had access to all clinical data. The interpretations were correlated with the results of surgical staging; however, a correlation of specific areas of Indium In 111 ProstaScint™ uptake to specific sites of tumor involvement was not performed.

One hundred fifty-two patients had an interpretable scan and surgical staging. Forty scans were classified as true positive, 25 as false positive, 63 as true negative, and 24 as false negative. The results for immunoscintigraphy are summarized in TABLE 4.

TABLE 4 - COMPARISON OF INDIUM IN 111 PROSTASCINT™ AND HISTOPATHOLOGIC RESULTS FOR PRESURGICAL PATIENTS

	Number of Patients: Indium In 111 ProstaScint™ +	Number of Patients: Indium In 111 ProstaScint™ −	
Biopsy +	40	24	*Sensitivity 62%*
Biopsy −	25	63	*Specificity 72%*
	Positive Predictive Value 62%	*Negative Predictive Value 72%*	*Overall Accuracy 68%*

Sixty-five patients (43%) had positive Indium In 111 ProstaScint™ images for pelvic lymph node metastases. Of these 38% (25 patients) did not have metastatic prostate cancer at surgery. Eighty-seven patients (57%) had negative Indium In 111 ProstaScint™ images. Of these 28% (24 patients) did have metastatic prostate cancer at surgery. The overall accuracy of Indium In 111 ProstaScint™ immunoscintigraphy, as measured against pelvic lymph node dissection, was 68% (103/152).

A retrospective subset analysis suggested that a positive Indium In 111 ProstaScint™ scan in patients with a Gleason score ≥7 and a PSA ≥ 40 contained additional information regarding the likelihood that tumor metastases would be found at the scheduled staging pelvic lymphadenectomy.

Imaging Performance in Patients with Occult Recurrent or Residual Disease

In the second open label, multi-center, uncontrolled pivotal phase 3 trial, 183 patients with a high clinical suspicion of residual or recurrent prostate cancer following radical prostatectomy were evaluated. Patients with a rising PSA, a negative bone scan, and negative or equivocal standard diagnostic techniques, (e.g. transrectal ultrasound, CT scan, or MRI) underwent Indium In 111 ProstaScint™ immunoscintigraphy prior to biopsy of the prostatic fossa. The Indium In 111 ProstaScint™ images were interpreted on-site, and the reader had access to all clinical data. The interpretations were correlated with the results of histopathologic analysis of the prostatic fossa biopsy specimens.

One hundred fifty-eight patients had an interpretable scan and prostatic fossa biopsy. Twenty-nine scans were classified as true positive, 29 as false positive, 70 as true negative, and 30 as false negative. The results are summarized in TABLE 5.

TABLE 5 - INDIUM IN 111 PROSTASCINT™ AND HISTOPATHOLOGIC RESULTS FOR RECURRENT OR RESIDUAL DISEASE PATIENTS

	Number of Patients: Indium In 111 ProstaScint™ +	Number of Patients: Indium In 111 ProstaScint™ −	
Biopsy +	29	30	*Sensitivity 49%*
Biopsy -	29	70	*Specificity 71%*
	Positive Predictive Value 50%	*Negative Predictive Value 70%*	*Overall Accuracy 63%*

Fifty-eight patients (37%) had positive Indium In 111 ProstaScint™ images in the prostatic fossa. Of these 50% (29 patients) did not have recurrent prostate cancer on biopsy. One hundred patients (63%) had negative Indium In 111 ProstaScint™ images. Of these 30% (30 patients) had recurrent prostate cancer on biopsy. The overall accuracy of Indium In 111 ProstaScint™ immunoscintigraphy, as measured against prostatic fossa biopsy, was 63% (99/158).

Indium In 111 ProstaScint™ localized to only the prostatic fossa in 29 (18%) patients, to prostatic fossa and extrafossa sites in 29 (18%) patients, and to only extrafossa sites in 39 (25%) patients. The study was not designed to evaluate extrafossa sites of uptake. Three extrafossa sites of uptake were biopsied, one of which was positive for metastatic prostate cancer.

ProstaScint™ Results in Patients with Distant Metastases

Clinical trials have not specifically studied the ability of Indium In 111 ProstaScint™ to image distant (extra-pelvic) metastases, and a limited number of patients with distant (primarily bone) metastases were enrolled. Thirteen patients out of 16 (81%) with CT evidence of distant soft tissue disease had positive extrafossa Indium In 111 ProstaScint™ scans. Thirty-five out of 61 patients (57%) with bone scan evidence of disease had positive Indium In 111 ProstaScint™ skeletal uptake; however, Indium In 111 ProstaScint™ imaging did not identify most sites of abnormal bone uptake on bone scan, nor did it demonstrate any new sites of metastasis that were not seen on bone scan. The Indium In 111 ProstaScint™ scan did, however, demonstrate sites of bone marrow metastases that were not seen on bone scan in 2 of 43 patients in the phase 1 study.

Repeat Scans

Sixty-one patients received a total of 74 repeat infusions of Indium In 111 ProstaScint™. The incidence of adverse reactions upon repeat infusion (5%) was comparable to that observed after single infusion (4 %). Human anti-mouse antibody (HAMA) levels were detected (at levels >8ng/mL) by radioimmune assay (RIA) after single infusion in 8% (20/239) of patients while 1% of patients had levels greater than 100 ng/mL. Serum HAMA levels were detected by RIA after repeat infusion in 19% (5/27) of patients.

Biodistribution was unaltered on 65 of 70 (93%) evaluable repeat scans. The efficacy of repeat Indium In 111 ProstaScint™ imaging was not evaluated.

INDICATIONS AND USAGE

Indium In 111 ProstaScint™ (Capromab Pendetide) is indicated as a diagnostic imaging agent in newly-diagnosed patients with biopsy-proven prostate cancer, thought to be clinically-localized after standard diagnostic evaluation (e.g. chest x-ray, bone scan, CT scan, or MRI), who are at high-risk for pelvic lymph node metastases (see CLINICAL PHARMACOLOGY, Imaging Performance in Newly-Diagnosed Patients). It is not indicated in patients who are not at high risk.

Indium In 111 ProstaScint™ is also indicated as a diagnostic imaging agent in post-prostatectomy patients with a rising PSA and a negative or equivocal standard metastatic evaluation in whom there is a high clinical suspicion of occult metastatic disease. The imaging performance of Indium In 111 ProstaScint™ following radiation therapy has not been studied.

The information provided by Indium In 111 ProstaScint™ imaging should be considered in conjunction with other diagnostic information. Scans that are positive for metastatic disease should be confirmed histologically in patients who are otherwise candidates for surgery or radiation therapy unless medically contraindicated. Scans that are negative for metastatic disease should not be used in lieu of histological confirmation.

ProstaScint™ is not indicated as a screening tool for carcinoma of the prostate nor for readministration for the purpose of assessment of response to treatment.

CONTRAINDICATIONS

Indium In 111 ProstaScint™ should not be used in patients who are hypersensitive to this or any other product of murine origin or to Indium In 111 chloride.

WARNINGS

Patient management should not be based on Indium In 111 ProstaScint™ (Capromab Pendetide) scan results without appropriate confirmatory studies since in the pivotal trials, there was a high rate of false positive and false negative image interpretations (See PRECAUTIONS).

Indium In 111 ProstaScint™ images should be interpreted only by physicians who have had specific training in Indium In 111 ProstaScint™ image interpretation (see PRECAUTIONS, Imaging Precautions).

Allergic reactions, including anaphylaxis, can occur in patients who receive murine antibodies. Although serious reactions of this type have not been observed in clinical trials after Indium In 111 ProstaScint™ administration, medications for the treatment of hypersensitivity reactions should be available during administration of this agent.

Indium In 111 ProstaScint™ may induce human anti-mouse antibodies which may interfere with some immunoassays, including those used to assay PSA and digoxin (see PRECAUTIONS, Drug/Laboratory Test Interactions).

PRECAUTIONS

General

There were high rates of false positive and false negative image interpretations in the pivotal trials (see Clinical Studies). False positive scan interpretations may result in: (1) inappropriate surgical intervention to confirm scan results; (2) inappropriate denial of curative therapy if results are not confirmed; or (3) inadequate surgical staging if only areas of uptake are sampled. Surgical sampling should not be limited to the areas of positive uptake, unless histologic examination of these areas is diagnostic. Due to the potential for false negative scan interpretations, negative images should not be used in lieu of histologic confirmation. Proper patient preparation is mandatory to obtain optimal images for interpretation (see Imaging Precautions, below).

Bone scans are more sensitive than ProstaScint™ (Capromab Pendetide) for the detection of metastases to bone, and Indium In 111 ProstaScint™ should not replace bone scan for the evaluation of skeletal metastases.

Imaging Precautions

Radiopharmaceuticals should be used only by physicians and other professionals who are qualified by training and experience in the safe use and handling of radionuclides. Indium In 111 ProstaScint™ images should be interpreted only by physicians who have had specific training in the interpretation of Indium In 111 ProstaScint™ images.

There may be Indium In 111 ProstaScint™ clearance and imaging localization observed in the bowel, blood pool, kidneys, and urinary bladder. When obtaining all 72-120 hour planar and Single-Photon Emission Computed Tomography (SPECT) images, the bladder should be catheterized and irrigated. The administration of a cathartic is required the evening before imaging the patient, and a cleansing enema should be administered within an hour prior to each 72-120 hour imaging session.

The contents of the kit are not radioactive. However, after the Indium In 111 chloride is added, appropriate shielding of Indium In 111 ProstaScint™ must be maintained. Care should be taken to minimize radiation exposure to patients and medical personnel, consistent with proper hospital and patient management procedures.
Each ProstaScint™ kit is a unit of use package. The contents of the kit are to be used only to prepare Indium In 111 ProstaScint™; unlabeled ProstaScint™ should NOT be administered directly to the patient. After radiolabeling with Indium In 111, the entire Indium In 111 ProstaScint™ dose must be administered to the patient for whom it was prescribed. Reducing the dose of Indium In 111 unlabeled ProstaScint™, or Indium In 111 ProstaScint™ may adversely impact imaging results and is not recommended. The components of the kit are sterile and pyrogen-free and contain no preservatives. Indium In 111 ProstaScint™ should be used within 8 hours after radiolabeling. It is essential to follow the directions for preparation carefully and to adhere to strict aseptic procedures during preparation of the radiolabeled product.

Information for Patients

Murine monoclonal antibodies (MAbs) are foreign proteins, and their administration can induce HAMA. While limited data exist concerning the clinical significance of HAMA, the presence of HAMA may interfere with murine-antibody based immunoassays, or could compromise the efficacy of diagnostic or therapeutic murine antibody-based agents and increase the risk of adverse reactions. For these reasons, patients should be informed that the use of this product could adversely affect the future ability to diagnose recurrence of their tumor, the ability to perform certain other laboratory tests, or to use other murine-based products. Patients should be advised to discuss prior use of murine-antibody based products with their physicians (see Heterologous Protein Administration, below).

Heterologous Protein Administration

Indium In 111 ProstaScint™ has been shown to induce HAMA to murine IgG infrequently and with low peak levels after single administration. HAMA levels were detected (at >8 ng/mL) by RIA after single infusion in 8% (20/239) of patients, while 1% of patients had levels greater than 100 ng/mL. In addition, serum HAMA levels were detected by RIA after repeat infusion in 19% (5/27) of the patients.
While limited data exist concerning the clinical significance of HAMA, detectable serum levels can alter the clearance and tissue biodistribution of MAbs. The development of persistently elevated serum HAMA levels could compromise the efficacy of diagnostic or therapeutic murine antibody-based agents. In repeat administration trials, 93% (65/70) of the evaluable repeat infusions were associated with normal tissue distribution of the MAb conjugate. Pre-infusion serum HAMA levels were generally not predictive of altered distribution.
When considering the administration of Indium In 111 ProstaScint™ to patients who have previously received other murine antibody-based products, physicians should be aware of the potential for assay interference and increased clearance and altered biodistribution, which may interfere with the quality or sensitivity of the imaging study. Prior to administration of murine antibodies, including Indium In 111 ProstaScint™, the physician should review the patient history to determine whether the patient has previously received such products.

Drug Interactions

The effect of surgical and/or medical androgen ablation on the imaging performance of Indium In 111 ProstaScint™ has not been studied. Preliminary data suggest hormone ablation may increase PSMA expression, with concurrent decrease in tumor expression of PSA. The use of ProstaScint™ in this patient population cannot be recommended at this time.

Drug/Laboratory Test Interactions

The presence of HAMA in serum as a result of ProstaScint™ may interfere with some antibody-based immunoassays (such as PSA and digoxin). When present, this interference generally results in falsely high values. When following PSA levels, assay methods resistant to HAMA interference should be utilized. PSA assays which were found to be resistant to HAMA interference were Hybritech Tandem-R and Abbott IMX.
When patients have received Indium In 111 ProstaScint™, the clinical laboratory should be notified to take appropriate measures to avoid interference by HAMA with clinical laboratory testing procedures. These methods include the use of non-murine-based immunoassays, HAMA removal by adsorption, or sample pre-treatment to block HAMA activity.

Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term animal studies have not been performed to evaluate the carcinogenic or mutagenic potential of Indium In 111 ProstaScint™ or to evaluate its effect on fertility.

Pregnancy

ProstaScint™ is not indicated for use in women.

Nursing Mothers and/or Lactating Women

ProstaScint™ is not indicated for use in women.

Pediatric Use

The safety and effectiveness of Indium In 111 ProstaScint™ in pediatric patients have not been established. ProstaScint™ is not indicated for use in children.

ADVERSE REACTIONS

ProstaScint™ (Capromab Pendetide) was generally well tolerated in the clinical trials. After administration of 529 single doses of Indium In 111 ProstaScint™, adverse reactions were observed in 4% of patients. The most commonly reported adverse reactions were increases in bilirubin, hypotension, and hypertension, which occurred in 1% of patients. Elevated liver enzymes and injection site reactions occurred in slightly less than 1% of patients. Other adverse reactions, listed in order of decreasing frequency, were: pruritus, fever, rash, headache, myalgia, asthenia, burning sensation in thigh, shortness of breath, and alteration of taste. Most adverse reactions were mild and readily reversible. Data from repeat administration in 61 patients revealed a similar incidence of adverse reactions (5%). No deaths were attributable to Indium In 111 ProstaScint™ administration.

OVERDOSAGE

The maximum amount of Indium In 111 ProstaScint™ (Capromab Pendetide) that can be safely administered has not been determined. In clinical studies, single doses of 10 mg of Indium In 111 ProstaScint™ were administered to 20 patients with prostate cancer; the type and frequency of adverse reactions at this dose were similar to those observed with lower doses. The maximum Indium In 111 dose administered with ProstaScint™ in a clinical study was 6.5 mCi.

DOSAGE AND ADMINISTRATION

The patient dose of the radiolabel must be measured in a dose calibrator prior to administration.
The recommended dose of ProstaScint™ (Capromab Pendetide) is 0.5 mg radiolabeled with 5 mCi of Indium In 111 chloride. Each dose is administered intravenously over 5 minutes and should not be mixed with any other medication during its administration. Indium In 111 ProstaScint™ may be readministered following infiltration or a technically inadequate scan; however, it is not indicated for readministration for the purpose of assessment of response to treatment (see INDICATIONS AND USAGE).
Each ProstaScint™ kit is a unit dose package. After radiolabeling with Indium In 111, the entire Indium In 111 ProstaScint™ dose should be administered to the patient. Reducing the dose of Indium In 111, unlabeled ProstaScint™, or Indium In 111 ProstaScint™ may adversely impact imaging results and is, therefore, not recommended. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit.

Radiation Dosimetry

The estimated absorbed radiation doses to an average adult patient from an intravenous injection of ProstaScint™ labeled with 5 mCi of Indium In 111 are shown in TABLE 6. Total dose estimates include absorbed radiation doses from both Indium In 111 and the Indium In 114m radiocontaminant. A level of 0.06% of Indium In 114m was utilized for the dose estimates presented in TABLE 6.

TABLE 6 - ESTIMATED AVERAGE ABSORBED RADIATION DOSE IN ADULT PATIENTS FROM INTRAVENOUS ADMINISTRATION OF PROSTASCINT™ LABELED WITH 5 mCi (185 MBq) OF Indium In 111 CHLORIDE[a]

Organ	Average Dose (rad/5 mCi)	Average Dose (mGy/ 185MBq)
Total body	2.7	27
Brain	1.1	11
Liver	18.5	185
Spleen	16.3	163
Kidneys	12.4	124
Lungs	5.6	56
Heart wall	7.8	78
Red marrow	4.3	43
Adrenals	5.2	52
Urine Bladder wall	2.2	22
Bone Surfaces	4.0	40
Stomach	3.1	31
Gall Bladder Wall	7.3	73
Small Intestine	3.3	33
Upper Large Intestine Wall	5.0	50
Lower Large Intestine Wall	7.6	76
Pancreas	5.1	51
Skin	1.1	11
Testes	5.6	56
Prostate	8.2	82
Thymus	2.6	26
Thyroid	1.4	14
Other Tissues	2.0	20

[a]Based on data from 21 patients who received doses of ProstaScint™ labeled with a mean (± SD) Indium In 111 dose of 4.6 ± 1.0 mCi.

Directions for Radiolabeling ProstaScint™ with Indium In 111 Chloride

Proper aseptic techniques and precautions for handling radioactive materials should be employed. Waterproof gloves should be worn during the radiolabeling procedure. The preparation of the product should be done by the following procedure.

1. Required materials, not supplied:
   A. Indium In 111 Chloride from Amersham Inc. Or Mallinckrodt, Inc.
   B. One sterile 1 mL syringe
   C. Vial shield
   D. Dose calibrator set for Indium In 111
   E. Gelman ITLC-GC strips
   F. Developing chamber for chromatography (e.g. scintillation vial)
   G. 21-23 gauge sterile needles
   H. Shield for 10 mL syringe
   I. Waterproof gloves
   J. Alcohol wipe
   K. Water-soluble marker
   L. 0.9% sodium chloride solution
   M. DTPA (0.05 M solution of diethylenetriamine pentaacetic acid)
   N. Gamma ray detector
2. Sterile, pyrogen-free Indium In 111 Chloride solution must be utilized in the preparation of the Indium In 111 ProstaScint™. The use of high purity Indium In 111 Chloride manufactured by Amersham, Inc. or by Mallinckrodt, Inc. is required. The Indium In 111 Chloride should be used only to radiolabel the ProstaScint™ and should not be injected directly into the patient. The Indium In 111 Chloride should not be utilized after its expiration date.
3. Before radiolabeling bring the refrigerated ProstaScint™ to room temperature. Note: ProstaScint™ is a protein solution which may develop translucent particulates. These particulates will be removed by filtration.
4. Clean the rubber stopper of each vial with an alcohol wipe. With a sterile 1 mL syringe add 0.1 mL of sodium acetate solution to the shielded vial of Indium In 111 chloride and mix. Retain remaining sodium acetate for use in Step 7.
5. With the same 1 mL syringe, withdraw between 6 & 7 mCi of the buffered Indium In 111 chloride and add to the ProstaScint™ vial. Flush the syringe to mix the preparation. To normalize pressure, withdraw an equal volume of air. Swirl gently to mix, and assay contents in a dose calibrator. On one of the labels provided, record the patient's identification, the date, time of preparation, and activity in the vial. Affix the label to the vial shield.
6. Allow the labeling reaction to proceed at room temperature for 30 minutes.
7. With a syringe, add the remaining sodium acetate to the ProstaScint™ reaction vial. To normalize pressure, withdraw an equal volume of air.
8. Aseptically attach the 0.22 μm Millex® GV sterile filter (provided) and a sterile hypodermic needle to a 10 mL sterile disposable syringe and withdraw the contents of the reaction vial through the filter into the syringe. Keep the needle immersed in the solution to avoid creating an air-lock in the filter.
9. Remove the filter and needle. Aseptically attach a fresh sterile hypodermic needle to the syringe. Assay syringe and contents in a dose calibrator. The syringe should contain not less than 4 mCi (148 MBq) of Indium In 111.
10. Radiochemical purity (RCP) by Instant Thin Layer Chromatography (ITLC) can be determined by the following procedure:
    A. Mix equal parts (several drops of each) of Indium In 111 ProstaScint™ with DTPA solution. Allow the mixture to stand at room temperature for one minute. Spot a small drop of the mixture onto an ITLC strip at its origin.
    B. Place the strip in a chromatography chamber with the origin at the bottom and allow the solvent to migrate 6 cm from the origin of the strip. Remove and cut the strip in half and measure the counts per minute (CPM) of both halves with a gamma ray detector.
    C. Calculate the percent RCP as follows:

$$\%RCP = \frac{\text{CPM bottom half}}{\text{CPM bottom half + CPM top half}} \times 100$$

    D. If the radiochemical purity is <90%, the ITLC procedure should be repeated. If repeat testing remains <90%, the preparation should not be administered.
11. On the second label provided in the kit, record the patient's identification, the date, time of assay, and activity in the syringe. Affix this label to the syringe shield.
12. Indium In 111 ProstaScint™ should be used within 8 hours of radiolabeling.
13. Discard vials, needles, and syringes in accordance with local, state, and federal regulations governing radioactive and biohazardous waste.

Image Acquisition and Interpretation

Images should be acquired using a large field of view gamma camera equipped with a parallel hole medium energy collimator. The gamma camera should be calibrated using the 172 and 247 keV photopeaks for Indium In 111 with a 20% symmetric window.
Whole body or spot planar views of the pelvis, abdomen, and thorax should be performed between 72 and 120 hours following Indium In 111 ProstaScint™ infusion. A cathartic is required the evening before imaging and a cleansing enema should be administered within an hour prior to each 72-120 hour imaging session. In addition, the bladder should be catheterized and irrigated.
Whole body acquisition should be carried out from skull through mid-femur. The total scan time over this area should be no less than 35 minutes using a 128x512 or 256x1024 matrix.
Planar images should be acquired in anterior and posterior views for 7.5 minutes per view using a 128x128 or 256x256 matrix. Due to uptake of Indium In 111 ProstaScint™ by the liver, planar images obtained with the liver in the field of view must be acquired with adequate counts to allow the detection of lesions in the adjacent extrahepatic abdomen and pelvis. This may result in pixel overflow with image degradation in the region of the liver.
Two SPECT imaging sessions are necessary. The first SPECT session should be of the pelvis and be performed approximately 30 minutes after infusion to obtain a blood pool image. The second SPECT session should include both the pelvis and abdomen, including the lower liver margin through the prostatic fossa and be performed between 72 and 120 hours after infusion for detection of benign and malignant prostate tissue sites. Depending upon the capability of the camera field of view to include both pelvis and abdomen, either one or two separate acquisitions may be necessary during the second session.
To resolve imaging ambiguities possibly resulting from activity in blood pool, stool or urinary bladder, follow-up imaging sessions with full patient preparation should be performed.
The SPECT Images should be acquired using a 64x64 or 128x128 matrix for a minimum of 60 or 120 stops, respectively, over 360 degrees rotation for approximately 25 seconds per view at the first session and 50 seconds per view at the second session. Reconstruction should be performed using a Butterworth filter or equivalent in the transverse, coronal and sagittal views. An order of 5 and cut off of 0.5 may be used as a starting point. Slice thickness should be in the range of 6 to 12 mm.
Following Indium In 111 ProstaScint™ administration, some of the radiolabel localizes in normal liver, spleen, bone marrow and genitalia.
It has been reported that Indium In 111 labeled antibodies may localize non-specifically in colostomy sites, degenerative joint disease, abdominal aneurysms, post-operative bowel adhesions, and local inflammatory lesions, including those typically associated with inflammatory bowel disease or secondary to surgery or radiation. Indium In 111 ProstaScint™ can demonstrate apparent localization to sites of tortuous blood vessels. Careful review of the patient's medical history and other diagnostic information should aid in the interpretation of the images.
The diagnostic images acquired with Indium In 111 ProstaScint™ should be interpreted in conjunction with other appropriate diagnostic tests.

HOW SUPPLIED

The ProstaScint™ (Capromab Pendetide) kit (NDC No. 57902-817-01) for the preparation of Indium In 111 labeled Capromab Pendetide includes one vial containing 0.5 mg of ProstaScint™ per 1 mL of sodium phosphate buffered saline and one 2 mL vial of sodium acetate solution, 0.5 *M*. These solutions are sterile and pyrogen free and contain no preservatives. Each kit also includes one sterile 0.22 μm Millex® GV filter, prescribing information, and two identification labels.

Storage

Store at 2° to 8°C (36° to 46°F). Do not freeze. Store upright.

REFERENCES

1. Kocher, DC: Radioactive decay data tables. *DOE/TIC* 115:11026, 1981.
2. Data supplied by Oak Ridge Associated Universities, Radiopharmaceutical Internal Dose Information Center, 1984.
3. Wright, GL, Jr; et al. Expression of Prostate-Specific Membrane Antigen in Normal, Benign, and Malignant Prostate Tissues. *Urol Oncol.* 1995; 1:18-28.

Manufactured and distributed by:
CYTOGEN Corporation
Princeton, NJ 08540-5308

Revised 10/28/96

Exhibit 2

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
1401 Rockville Pike
Rockville MD 20852-1448

October 28, 1996

Our Reference No. 95-0041 and 95-0087

Mr. Michael A. Trapani
Cytogen Corporation
600 College Road East
Princeton, NJ 08540

Dear Mr. Trapani:

Your biologics license application for Capromab Pendetide is approved effective this date. Cytogen Corporation, Princeton, New Jersey is hereby authorized to manufacture and ship for sale, barter, or exchange in interstate and foreign commerce Capromab Pendetide under Department of Health and Human Services Biologics License No. 1164.

Capromab Pendetide is indicated for the preparation of Indium In 111 Capromab Pendetide to be used as a diagnostic imaging agent in newly diagnosed patients with biopsy-proven prostate cancer, thought to be clinically localized after standard diagnostic evaluation, who are at high risk for pelvic lymph node metastases, and in post-prostatectomy patients with a rising PSA and a negative or equivocal standard metastatic evaluation in whom there is a high clinical suspicion of occult metastatic disease.

In accordance with approved labeling, your product will bear the tradename ProstaScint, and will be marketed as a single dose kit containing a 1 mL vial of Capromab Pendetide solution and a 1 mL vial of sodium acetate buffer solution.

You are not currently required to submit samples of future lots of Capromab Pendetide to the Center for Biologics Evaluation and Research (CBER) for release by the Director, CBER, under 21 CFR 610.2. FDA will continue to monitor compliance with 21 CFR 610.1 requiring assay and release of only those lots that meet release specifications.

The dating period for this product shall be 24 months from the date of manufacture when stored at 2-8° C. The date of manufacture shall be defined as the date of final sterile filtration of the formulated product. The bulk Capromab antibody may be stored for up to 36 months at 2-8°C or -70°C. Results of ongoing stability studies should be submitted throughout the dating period as they become available including the results of stability studies from the first three production lots.

We acknowledge your written commitment of October 25, 1996 to submit a galley proof of the package insert for review and approval prior to implementation.

Any changes in the manufacture, packaging or labeling of the product or in the manufacturing facilities will require the submission of information to your biologics license application for our review and written approval consistent with 21 CFR 601.12.

It is requested that adverse experience reports be submitted in accordance with the adverse experience reporting requirements for licensed biological products (21 CFR 600.80) and that distribution reports be submitted as described (21 CFR 600.81). These requirements became effective on December 27, 1994. All adverse experience reports should be prominently identified according to 21 CFR 600.80 and be submitted to the Center for Biologics Evaluation and Research, HFM-210, Food and Drug Administration, 1401 Rockville Pike, Rockville, MD 20852-1448.

Please submit three copies of all final printed labeling at the time of use and include part II of the label transmittal form (FDA Form 2567) with completed implementation information. In addition, you may wish to submit draft copies of the proposed introductory advertising and promotional labeling with an FDA Form 2567 to the Center for Biologics Evaluation and Research, Advertising and Promotional Labeling Staff, HFM-202, 1401 Rockville Pike, Rockville, MD 20852-1448. Final printed advertising and promotional labeling should be submitted at the time of initial dissemination, accompanied by an FDA Form 2567. All promotional claims must be consistent with and not contrary to approved labeling. No comparative promotional claim or claim of superiority over other similar products should be made unless data to support such claims are submitted to and approved by the Center for Biologics Evaluation and Research.

Sincerely yours,

Jay P. Siegel, M.D., FACP
Director
Office of Therapeutics
Research and Review
Center for Biologics
Evaluation and Research

Jerome A. Donlon, M.D., Ph.D.
Director
Office of Establishment Licensing
and Product Surveillance
Center for Biologics
Evaluation and Research

Exhibit 3

US005162504A

United States Patent [19]

Horoszewicz

[11] Patent Number: 5,162,504

[45] Date of Patent: Nov. 10, 1992

[54] MONOCLONAL ANTIBODIES TO A NEW ANTIGENIC MARKER IN EPITHELIAL PROSTATIC CELLS AND SERUM OF PROSTATIC CANCER PATIENTS

[75] Inventor: Julius S. Horoszewicz, Williamsville, N.Y.

[73] Assignee: Cytogen Corporation, N.J.

[21] Appl. No.: 202,869

[22] Filed: Jun. 3, 1988

[51] Int. Cl.[5] C07K 15/28; C12N 5/20; C12P 21/08

[52] U.S. Cl. 530/388.2; 435/240.27; 435/70.21; 435/7.23; 530/388.8

[58] Field of Search 530/387; 435/70.21, 435/240.27, 7, 7.23

[56] References Cited

PUBLICATIONS

Wright et al., Cancer Res. 43:5509-16, 1983.
Horoszewicz et al., Anticancer Res. 7:927-36, 1987.
Frankel et al., PNAS 79:903-907, 1982.
Webb et al., Cancer Immunol Immunother 17:7-17, 1984.
Finstad et al., PNAS 82:2955-59, 1985.
Campbell "Monoclonal Antibody Technology" Elsevier Publ. 1984, 265 pages.

Primary Examiner—John Doll
Assistant Examiner—Paula Hutzell
Attorney, Agent, or Firm—Pennie & Edmonds

[57] ABSTRACT

Monoclonal antibodies to prostatic cells, are produced by a hybridoma formed by fusing mouse lymphocytes and mouse myeloma cells. The monoclonal antibodies show specificity for a non-soluble, membrane associated, organ specific antigenic determinant limited in its distribution to normal and neoplastic, human prostate epithelial cells. The monoclonal antibodies, specifically 7E11-C5 monoclonal antibodies, may be suitable for diagnostic uses.

2 Claims, 3 Drawing Sheets

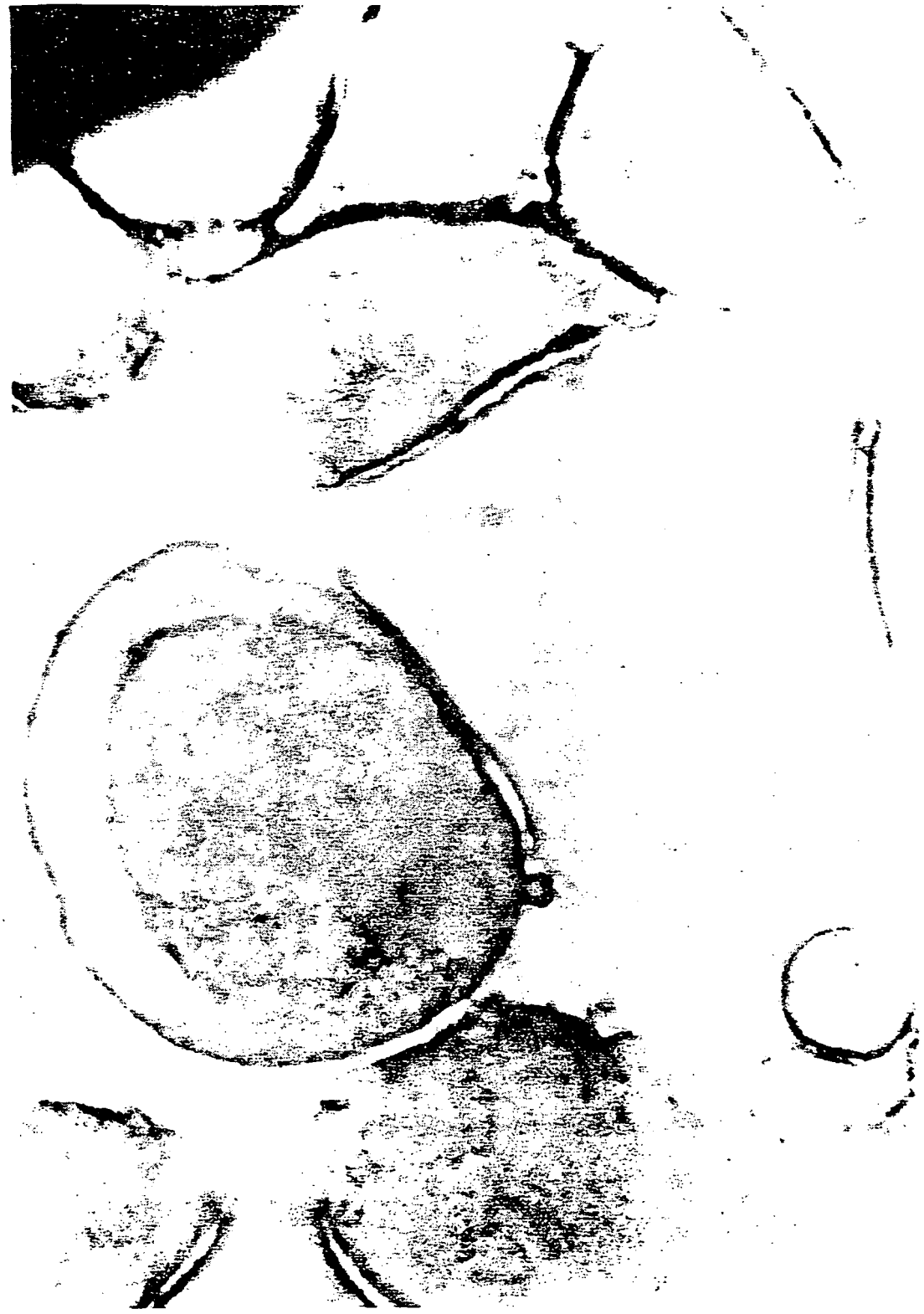

FIG. 2

MONOCLONAL ANTIBODIES TO A NEW ANTIGENIC MARKER IN EPITHELIAL PROSTATIC CELLS AND SERUM OF PROSTATIC CANCER PATIENTS

TABLE OF CONTENTS

1. INTRODUCTION

This invention relates to the production of and applications for monoclonal antibodies specific for prostatic tumor antigens. More particularly, this invention relates to monoclonal antibodies against non-soluble, membrane associated, organ specific determinants expressed maximally on human normal and neoplastic prostatic epithelium. Monoclonal antibodies capable of reacting with membrane associated surface antigens are of value for the immuno-classification and detection of disease and represent novel agents for immunotherapy. The monoclonal antibodies of this invention possess distinctive characteristics and capabilities which make them suitable for in vitro clinical diagnostic and prognostic purposes. Moreover, they are of great potential importance for in vivo tumor localization and cancer therapy in humans.

The monoclonal antibodies exhibit a high level of binding to human prostatic cancer cells and normal prostatic epithelium and are potentially capable of experimental in vivo tumor localization. They bind to well-differentiated as well as to poorly-differentiated tumors.

The invention provides methods for production of the monoclonal antibodies by hybridoma techniques. Once cloned, cell lines can be maintained continuously to produce an unlimited homogeneous monoclonal antibody population that can be isolated and/or purified and used clinically for in vitro immunohistological, immuno-cytological or immuno-serological diagnosis, in vivo diagnosis by localization of tumors and metastases, and immunotherapy of human cancers, particularly those of the prostate.

2. BACKGROUND OF THE INVENTION

2.1. Monoclonal Antibodies

Kohler and Milstein are generally credited with having devised the techniques that successfully resulted in the formation of the first monoclonal antibody-producing hybridomas [Kohler. G. and Milstein, C., Nature 256:495–497 (1975); Eur. J. Immunol. 6:511–519 (1976)]. By fusing antibody-forming cells (spleen lymphocytes) with myeloma cells (malignant cells of bone marrow primary tumors) they created a hybrid cell line, arising from a single fused cell hybrid (called a hybridoma or clone) which had inherited certain characteristics of both the lymphocytes and myeloma cell lines. Like the lymphocytes (taken from animals primed with sheep red blood cells as antigen), the hybridomas secreted a single type of immunoglobulin specific to the antigen; moreover, like the myeloma cells, the hybrid cells had the potential for indefinite cell division. The combination of these two features offered distinct advantages over conventional antisera. Whereas antisera derived from vaccinated animals are variable mixtures of polyclonal antibodies which never can be reproduced identically, monoclonal antibodies are highly specific immunoglobulins of a single type. The single type of immunoglobulin secreted by a hybridoma is specific to one and only one antigenic determinant, or epitope, on the antigen, a complex molecule having a multiplicity of antigenic determinants. For instance, if the antigen is a protein, an antigenic determinant may be one of the many peptide sequences [generally 6–7 amino acids in length (Atassi, M. Z., Molec. Cell. Biochem. 32:21–43 (1980))] within the entire protein molecule. Hence, monoclonal antibodies raised against a single antigen may be distinct from each other depending on the determinant that induced their formation; but for any given clone, all of the antibodies it produces are identical. Furthermore, the hybridoma cell line can be reproduced indefinitely, is easily propagated in vitro or in vivo, and yields monoclonal antibodies in extremely high concentration.

2.2. APPLICATION OF MONOCLONAL ANTIBODIES TO CANCER

Monoclonal antibodies produced by hybridoma technology are potentially powerful tools for cancer detection, diagnosis and therapy. [For a general discussion of the topic, see Hybridomas in Cancer Diagnosis and Treatment, Mitchell, M. S. and Oettgen, H. F., (eds.), Progress in Cancer Research and Therapy, Vol. 21, Raven Press, New York (1982)]. It has been reported that monoclonal antibodies have been raised against tumor cells [U.S. Pat. No. 4,196,265], carcinoembryonic antigen [U.S. Pat. No. 4,349,528], and thymocytes, prothymocytes, monocytes and suppressor T cells [U.S. Pats. Nos. 4,364,933; 4,364,935; 4,364,934; 4,364,936;

Starling, J. et al. (Canc. Res. 42:3084–3089 (1982) reported that a short immunization schedule with DU-145 cells (days 0, 8 and 15) of BALB/c mice resulted in isolation of monoclonal antibody 83.21 which bound to surfaces of DU-145 cells. This monoclonal antibody was of the IgM class and did not bind to a variety of human tumors, normal tissues, several cell lines, nor to normal human prostatic epithelium. Membrane preparations from one metastatic CaP, PC-3 and DU-145 cells efficiently bound monoclonal antibody 83.21. The spectrum and degree of reactivities of this monoclonal antibody with different cells varied depending upon the antibody binding assays used such as immunofluorescence, complement dependent cytotoxicity or quantitative adsorption analysis. Epitopes detected by monoclonal antibody 83.21 were present on 58% primary CaP and, 17% CaP metastases. In addition significant cross-reactions with transitional cell carcinoma of the bladder, cytomegalovirus transformed human embryonic cell line as well as with proximal convoluted tubules of normal kidney were seen.

Wright, G. et al. [Canc. Res. 53:5509–5516 (1983)] immunizing mice with PC-3 cells obtained monoclonal antibody P 6.2. This antibody, also of the IgM class, reacted with 72% of paraffin embedded specimens of CaP, however lung cancer, breast cancer, pancreas cancer and human normal kidney also stained.

Ware, J. et al. [Canc. Res. 42:1215–1222 (1982)] produced monoclonal antibody alpha Pro-3 of the IgG2a sub-class by immunizing mice with PC-3 cells. This antibody recognized an antigen (p54) concentrated in human primary prostatic carcinoma removed surgically. The antigen p54 is also present in variable amounts in extracts from benign prostatic hypertrophy (BPH), testicular tumors, kidney cancer, thyroid cancer, bladder cancer, ovarian cancer and in normal non-prostatic tissues. Cultured cells such as PC-3, human breast carcinoma (MDA-MB-231) and normal human fibroblasts (IMR90) all show surface binding of monoclonal antibody Pro-3. Binding of alpha Pro-3 to another CaP derived cell line, DU-145 is only minimal. Another epitope of the p54 antigen is also recognized by monoclonal antibody alpha Pro-5.

Short term immunization with a mixture of three cell lines derived from human CaP led to the isolation of monoclonal antibody alpha Pro-13 by Webb, K. et al. [Canc. Immunol. Immunother. 17:7–17 (1984)]. In the solid phase binding assay this antibody reacted with 8 out of 10 extracts of CaP and benign prostate hypertrophy, but bladder cancer and kidney cancers were also positive, though to a smaller degree. Immunoperoxidase staining of frozen sections produced positive staining in epithelial cells in 4 out of 12 CaP and in 1 out of 6 BPH specimens. Cross-reactivity with non-prostatic tissue occurred with renal carcinoma, glands of the normal trachea, and vessel endothelium from testis and tonsils. Among cultured cells, PC-3, lung cancer, colon cancer and melanoma contained surface molecules recognized by alpha Pro-13. The antigen defined by monoclonal antibody alpha Pro-13 is a glycoprotein of 120,000 molecular weight (nonreduced) which is intrinsically stable on the cell surface with negligible release in cell culture supernatant or solubilized significantly only after CHAPS, but not Triton X-100 detergent treatment.

Several monoclonal antibodies reactive with surface components of normal and malignant human prostatic epithelium were obtained by Frankel, A. [Proc. Natl. Acad. Sci. USA, 79:903–907 (1982)] who used membrane enriched fractions from benign prostatic hypertrophy for immunization. All of them exhibited significant cross-reactivity with either kidney, spleen, thymus, pancreas, bladder, lung thyroid or brain tissue.

Carroll, A. et al. [Clin. Immunol, Immunopath. 33:268–281 (1984)] raised monoclonal antibodies to PC-3 cells. One hybridoma—F77-129 (IgG3 subclass) reacted with prostatic cancer cell lines (PC-3 and DU-145), 3 out of 4 breast cancer cell lines and one colon cancer line. Immunoperoxidase staining of human tissues confirmed binding to normal and malignant human prosatic and breast tissue. Radioiodinated F77-129 localized readily in tumors induced by injection of PC-3 cells into nude mice.

There is a need for monoclonal antibodies which are prostate specific and which will not cross react with other tissue types. The use of such antibodies can add significant information regarding functional classifications of individual prostate tumors to augment clinical classifications.

The pattern of staining for the monoclonal antibody of this invention is distinct from the reactivities of previously described monoclonal antibodies which recognize antigens expressed by prostate tumors. The monoclonal antibodies provided herein stain malignant prostate epithelial cells intensely, mostly on the periphery of cells with a small degree of heterogeneity. Normal prostate epithelial cells or benign prostatic hypertrophy cells showed either faint or only a moderate degree of staining. By comparison with the monoclonal antibodies described by Frankel (supra), the monoclonal antibodies of the invention do not stain non-epithelial components of the prostate (fibers, muscle, stroma, vessels, etc.). Additionally no specific staining was observed in non-prostatic malignant tumors.

3. SUMMARY OF THE INVENTION

Prior to the present invention, applicant believes that a non-soluble, prostate cancer specific antigen has not been defined by monoclonal antibodies, although some cancer of the prostate-associated epitopes have been identified.

The present invention provides methods and compositions for producing novel monoclonal anti-prostate carcinoma antibodies with specific binding capabilities and encompasses the used said antibodies for cancer immunodiagnosis, prognosis and therapy in humans. Specifically, the invention provides novel hybridoma-derived monoclonal antibodies which demonstrate a narrow spectrum of organ-specific reactivity with non-soluble, membrane associated antigenic determinants (epitopes) present on normal neoplastic and malignant human prostatic epithelium. The monoclonal antibodies do not react specifically with non-prostatic tumors and other tissues. The monoclonal antibodies stain malignant prostatic cells intensely and non-malignant prostatic epithelium weakly.

In addition, to their use as in vitro immuno-histological reagents for cancer diagnosis, the present invention contemplates the use of the monoclonal antibodies for in vivo diagnosis. Because of their ability to target prostate carcinoma cells, the monoclonal antibodies can be used in tumor localization and in the monitoring of metastases.

The invention further contemplates the use of the monoclonal antibodies provided herein as a diagnostic and prognostic tool for detection of cancer of the pros-

Ag4/1, MPC11-45.6TG1.7, C63-Ag8.653, Sp2/0-Ag14, FO, and S194/5XX0.BU.1, all derived from mice, 210.RCY3.Agl.2.3 derived from rats and U-226AR, and GM1500GTGAL2, derived from rats and U-226AR, and GM1500GTGAL2, derived from humans, [G. J. Hammerling, U. Hammerling and J. F. Kearney (editors), Monoclonal Antibodies and T-cell Hybridomas IN: J. L. Turk (editor) Research Monographs in Immunology, Vol. 3, Elsevier/North Holland Biomedical Press, New York (1981)].

5.4. FUSION

Methods for generating hybrids of antibody-producing spleen or lymph node cells and myeloma cells usually comprise mixing somatic cells with myeloma cells in a 2:1 proportion as in the example in Section 6.2. (though the proportion may vary from about 20:1 to about 1:1), respectively, in the presence of an agent or agents (chemical or electrical) that promote the fusion of cell membranes. It is often preferred that the same species of animal serve as the source of the somatic and myeloma cells used in the fusion procedure. Fusion methods have been described by Kohler and Milstein [Nature 256:495–497 (1975) and Eur. J. Immunol. 6:511–519 (1976)], and by Gefter et al. [Somatic Cell Genet. 3:231–236 1977)]. The fusion-promotion agent used by those investigators were Sendai virus and polyethylene glycol (PEG), respectively. The fusion procedure of the example of the present invention is a modification of the method of Kohler and Milstein, supra.

5.5. ISOLATION OF CLONES AND ANTIBODY DETECTION

Fusion procedures usually produce viable hybrids at very low frequency, about 1×10^{-6} to 1×10^{-8}. Because of the low frequency of obtaining viable hybrids, it is essential to have a means to select fused cell hybrids from the remaining unfused cells, particularly the unfused myeloma cells. A means of detecting the desired antibody-producing hybridomas among the other resulting fused cell hybrids is also necessary.

Generally, the fused cells are cultured in selective media, for instance HAT medium containing hypoxanthine, aminopterin and thymidine. HAT medium permits the proliferation of hybrid cells and prevents growth of unfused myeloma cells which normally would continue to divide indefinitely. Aminopterin blocks de novo purine and pyrimidine synthesis by inhibiting the production of tetrahydrofolate. The addition of thymidine bypasses the block in pyrimidine synthesis, while hypoxanthine is included in the media so that inhibited cells synthesize purine using the nucleotide salvage pathway. The myeloma cells employed are mutants lacking hypoxanthine phosphoribosyl transferase (HPRT) and thus cannot utilize the salvage pathway. In the surviving hybrid, the B lymphocyte supplies genetic information for production of this enzyme. Since B lymphocytes themselves have a limited life span in culture (approximately two weeks), the only cells which can proliferate in HAT media are hybrids formed from myeloma and spleen cells.

To facilitate screening of antibody secreted by the hybrids and to prevent individual hybrids from overgrowing others, the mixture of fused myeloma and B lymphocytes is diluted in HAT medium and cultured in multiple wells of microtiter plates. In two to three weeks, when hybrid clones become visible microscopically, the supernatant fluid of the individual wells containing hybrid clones is assayed for specific antibody. The assay must be sensitive, simple and rapid. Assay techniques include radioimmunoassays, enzyme immunoassays, cytotoxicity assays, plaque assays, dot immunobinding assays, and the like.

5.6. CELL PROPAGATION AND ANTIBODY PRODUCTION

Once the desired fused cell hybrids have been selected and cloned into individual antibody-producing cell lines, each cell line may be propagated in either of two standard ways. A sample of the hybridoma can be injected into a histocompatible animal of the type that was used to provide the somatic and myeloma cells for the original fusion. The injected animal develops tumors secreting the specific monoclonal antibody produced by the fused cell hybrid. The body fluids of the animal, such as serum or ascites fluid, can be tapped to provide monoclonal antibodies in high concentration. Alternatively, the individual cell lines may be propogated in vitro in laboratory culture vessels; the culture medium, also containing high concentrations of a single specific monoclonal antibody, can be harvested by decantation, filtration or centrifugation.

5.7. IN VITRO DIAGNOSTIC USES FOR MONOCLONAL ANTIBODIES TO HUMAN PROSTATE CANCER

5.7.1. Immunohistological and Immuncytological Applications

The monoclonal antibodies of this invention can be used as probes in detecting discrete antigens in human tumors. The expression or lack of expression of these antigens can provide clinically exploitable information which is not apparent after standard histopathological evaluations. It may thus be possible to correlate the immuno-phenotypes of individual tumors with various aspects of tumor behavior and responsiveness to certain types of therapies, thus establishing important classifications of prognosis.

Monoclonal antibodies produced by the hybridoma methodologies herein described can be used to detect potential prostate carcinoma cells in histological and cytological specimens and in particular, to distinguish malignant from non-malignant tumors based on staining patterns and intensities. For instance, using the immunoperoxidase staining technique described in section 6.7., it has been observed that the monoclonal antibodies of its invention stained neoplastic prostate cells, mostly on the periphery of cells, with a small degree of heterogeneity. Morphologically nonmalignant prostatic ductal epithelium from benign prostatic hypertrophy and normal prostates generally exhibited a reduced degree of staining with cellular localization similar to prostatic cancer cells. Staining was completely absent from non-epithelial components of the prostate; no specific staining was observed in non-prostatic malignant tissues nor in normal human organs and tissues examined.

As an alternative to immunoperoxidase staining, immunofluorescent techniques can be used to examine human specimens with monoclonal antibodies to prostate carcinoma. In a typical protocol, slides containing cryostat sections of frozen, unfixed tissue biopsy samples or cytological smears are air dried formalin fixed and incubated with the monoclonal antibody preparation in a humidified chamber at room temperature.

forming an aldehyde which is then reacted with the antibody molecule. Attachment occurs via formation of a Schiff base with amino groups of the antibody molecule. Additionally, drugs with reactive sulfhydryl groups have been coupled to antibody molecules.

Similarly, glycosidic enzymes such as neuraminidase or α-mannosidase can be conjugated to the monoclonal antibodies. Conjugated antibodies can be administered to patients to achieve enhanced tumoricidal effects through the cytotoxic action of the chemotherapeutic agents or the increased binding effect of the glycosidic enzymes.

6. EXAMPLES

6.1. Cell Lines and Tissues

The LNCaP cell line was established from a metastatic lesion of human prostatic carcinoma. The LNCaP cells grow readily in vitro (up to 8×10^5 cells/ cm^2; doubling time, 60 hours), form clones in semisolid media, and show an aneuploid (modal number, 76 to 91) human male karyotype with several marker chromosomes. The malignant properties of LNCaP cells are maintained. Athymic nude mice develop tumors at the injection site (volume-doubling time, 86 hours). Functional differentiation is preserved; both cultures and tumor produce a prostate acid phosphatase (PAP) and prostate specific antigen (P.S.A.). High-affinity specific androgen receptors are present in the cytosol and nuclear fractions of cells in culture and in tumors. Estrogen receptors are demonstrable in the cytosol. The model is hormonally responsive. In vitro, α-dihydrotestosterone modulates cell growth and stimulates acid phosphatase production. In vivo, the frequency of tumor development and the mean time of tumor appearance are significantly different for either sex. LNCaP cells, therefore, meet criteria of a versatile model for immunological studies of human prostatic cancer in the laboratory.

Seven malignant cell lines of human origin were obtained from J. Fogh of Memorial Sloan-Kettering Institute and included: DU-145 and PC-3 derived from prostatic cancer; MCF-7, derived from pleural effusion of scirrhous carcinoma of the breast [Soule, D. G. et al., J. Natl. Cancer Inst., 51:1409–1416 (1973)]; MeWo, malignant melanoma; RT-4, transitional cell carcinoma; HT-29, adenoma of the colon and A209, rhabdomyosarcoma. Four other cell lines (two malignant and two normal), isolated and established at Roswell Park Memorial Institute were also used: TT, thyroid medullary carcinoma, pancreatic cancer, BG-9 and MLD—both normal diploid neonatal foreskin fibroblast (see Horoszewicz et al., Infect. Immun. 19:720–726, 1978; Chen et al., Human Pancreatic Adenocarcinoma, Vol. 18: 24–32, 1982; Leong et al., Advances in Thyroid Neoplasia 1984:95–108, 1982). All of the above cell lines were routinely maintained in RPMI medium 1640 (Roswell Park Memorial Institute, Buffalo, N.Y.) supplemented with 10% heat inactivated fetal bovine serum, 1 mM L-glutamine, and 50 μg/ml of penicillin and streptomycin (Gibco, Grand Island, N.Y.).

Fresh normal, benign and malignant prostate cancer tissues were obtained either from the Department of Surgery or the Department of Pathology at Roswell Park Memorial Institute. The tissues were quick frozen in M-1 embedding matrix (Lipshaw Corp., Detroit, Mich.) and stored at −80° C.

6.2. IMMUNIZATION AND CELL FUSION

Ten week old male Balb/c mice (West Seneca Laboratory, West Seneca, N.Y.) received intraperitoneal injections (2×10^7 cells/0.2 ml) of washed (3 times in RPMI medium 1640, Roswell Park Memorial Institute, Buffalo, N.Y.) live LNCaP cells suspended in RPMI medium 1640, at monthly intervals for 3 months. Three days before fusion, the mice received an intraperitoneal challenge of 2×10^7 cells in RPMI medium 1640 and an intravenous injection of the plasma membrane isolated from 1×10^8 LNCaP cells. Cell fusion was carried out using a modification of the procedure developed by Kohler and Milstein [Nature (Lond.) 256:495–497 (1975)]. Mouse splenocytes (1×10^8 cells) were fused in HyBRL-Prep 50% polyethylene glycol 1450, Bethesda Research Laboratories, Inc., Gaithersburg, Md.) with 5×10^7 mouse myeloma cells (P3×63Ag8.653). Fused cells were distributed to ten 96-well culture plates (Falcon, Oxnard, Calif.) and grown in hypoxanthine/aminopterin/thymidine (HAT) medium at 37° C. with 7.5% CO_2 in a humid atmosphere. Fourteen days later, supernatants were assayed for binding activity to plasma membrane isolate from LNCaP cells and MLD (normal human fibroblasts) using the Enzyme Linked Immunosorbent Assay (ELISA) with anti-mouse IgG β-galactosidase linked $F(ab')_2$ fragment from sheep (Amersham Corp., Amersham, England) or goat anti-mouse IgG horseradish peroxidase conjugate (Bio-Rad Laboratories, Richmond, Calif.) in a primary screen. Dried membrane isolate (400 ng/well) instead of whole LNCaP cells was used in the primary screening process because of poor attachment of the LNCaP cells to the plastic wells. To circumvent this problem, immunofiltration on a disposable microfold system (V & P Scientific, San Diego, Calif.) using whole LNCaP cells was used as a confirmatory assay as described in Section 6.5. In addition, the dot-immunobinding assay on nitrocellulose membrane (Section 6.4) was used to screen the supernatants of hybridomas for reactivity against LNCaP cell cytosol (100,000×g supernatants) and crude plasma membrane preparation. To determine the specificity spectrum of the cultures showing reactivity with the plasma membranes and/or whole LNCAP cells, the culture fluids were further tested by ELISA on a panel of an additional 9 viable, normal and neoplastic human cells lines as described in Section 6.5.

6.3. ISOLATION OF PLASMA MEMBRANE-ENRICHED FRACTION

Plasma membrane-enriched fractions were obtained from LNCaP cells and normal human diploid fibroblast strain MLD by modification of published methods [Kartner, N. et al., J. Membrane Biol. 36:191–211 (1977)]. Briefly, MLD cells in roller bottles or LNCaP cells in plastic culture flasks were gently rinsed 4 times with phosphate buffered saline (PBS). The cells were then rinsed once with hypotonic lysing buffer (3 mM Hepes [hydroxyethylpiperazine-ethanesulfonic acid], pH 7.0, 0.3 mM $MgCl_2$, 0.5 mM $CaCl_2$) and the buffer discarded. Fresh lysing buffer (5–25 ml) was added to each bottle or flask and the cells allowed to swell for 30 minutes at room temperature. The swollen cells were removed from the surface and disrupted by manual shaking. The progress of disruption was monitored by phase microscopy of a sample droplet. Gentle trituration (8–10 times) with a 10 ml pipette was used to complete disruption of the LNCaP cells. Vigorous shaking

Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1980)]. Stable cultures of antibody-producing hybridomas were expanded in complete media [RPMI 1640 media supplemented with 10% (w/v) heat-inactivated fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10 μg/ml insulin (GIBCO) and cryopreserved. After cloning, two stable monoclonal hybridoma cell lines were obtained and designated as 7E11-C5 and 9H10-A4 respectively.

Exhausted culture fluids and mouse ascites fluids were the source of antibodies used for further studies. Ascites fluid from mice carrying the hybridoma cell line was used to obtain large quantities of monoclonal antibodies. Hybridoma cells for ascites fluid production were washed 2 times with RPMI 1640 medium and resuspended at a density of $1-5\times10^7$ cells/ml. Using a 20-gauge needle, 0.2 ml of the cell suspension was injected into the peritoneal cavity of female nude mice. Pristane was not routinely used to precondition the animals. Ascites fluid containing high titers of antibodies was regularly harvested 4–5 weeks after injection with the hybridoma cells.

6.6. ISOTYPING OF MONOCLONAL ANTIBODIES

Monoclonals 7E11-C5 and 9H10-A4 are of the IgG1 subclass, as determined by double diffusion gel precipitation with isotype specific antisera (Miles). Consistent with this finding were observations that Protein A conjugated with either fluoroscein or horseradish peroxidase (BIO-RAD) failed to react with smears of LNCaP cells following incubation with either monoclonal.

6.7. INDIRECT IMMUNOPEROXIDASE STAINING OF TUMOR SPECIMENS OF MONOCLONAL ANTIBODIES 7E11C5

In a first set of experiments, cytospin smears of cultured cells, formalin-fixed cryostat (−25° C.) sections and sections of formalin fixed, paraffin embedded human tissues were used for immunoperoxidase staining as described previously [Heyderman E. and Neville, A. M., J. Clin. Path., 30:138–140 (1976)]. Briefly, hydrated paraffin tissue section or formalin fixed cryostat sections and cytospin smears (2% paraformaldehyde, pH 7.5, 60 minutes, at room temperature) were treated with 10% pooled normal human serum in PBS and washed for 15 minutes in PBS containing 0.5% Nonidet P-40. The sections were then incubated (60 min., 37° C.) with monoclonal antibody preparations (30 μl, 5–20 μg/ml) diluted in PBS containing 1% w/v BSA (Sigma). Following 4 washes in PBS, peroxidase conjugated goat antibodies against murine immunoglobulin (1:50 dilution in bovine serum albumin (Bio-Rad) were applied for 60 minutes at 37° C. After thorough washing in PBS (4 changes), the peroxidase activity was revealed using diaminobenzidine 0.5 mg/ml): H_2O_2 (0.01%) substrate in 0.1M Tris buffer pH 7.2 The sections were dehydrated in increasing concentrations of ethanol to xylene and then mounted in Permount (Fisher Scientific, Fairlawn, N.J.). In addition to the normal controls, control experiments which included PBS in place of the primary antibody, peroxidase-conjugated antibody alone and culture fluid from the myeloma cell line P3×63Ag8.653 were used. The intensity of the immunoreaction product was evaluated using a Zeiss microscope (40×objective; 10×ocular), and scored using a(−) to (+++) scale.

The reactivity of the two stable monoclonal antibodies with LNCaP cells, as well as 32 other human normal and malignant cell lines, is presented in Table I.

TABLE I

REACTIVITY OF MoAb 7E11-C5 AND MoAb 9H10-A4 WITH CULTURED HUMAN CELLS BY ELISA AND IMMUNOPEROXIDASE STAINING

Human Cells in Culture		Reactivity with MoAb 7E11-C5	Reactivity with MoAb 9H10-A4
LNCaP	Prostatic Ca	+++	++++
DU145	Prostatic Ca	—	—
PC-3	Prostatic Ca	—	—
RT-4	Bladder Ca	—	—
5637	Bladder Ca	—	—
MCF-7	Breast Ca	—	—
MDA-MB-231	Breast Ca	—	—
HT-29	Colon Ca	—	—
SK	Colon Ca	—	—
COLO205	Colon Ca	—	—
PAC	Pancreatic Ca	—	—
TT	Medullary Thyroid Ca	—	—
MeWo	Melanoma	—	—
SM	Melanoma	—	—
HeLa-531	Uterine Ca	—	—
HeLa-CCL2	Uterine Ca	—	—
A209	Rhabdomysarcoma	—	—
SW872	Liposarcoma	—	—
HT1080	Fibrosarcoma	—	—
5959	Osteogenic Sarcoma	—	—
SAOS-2	Osteogenic Sarcoma	—	—
HBC	Bronchogenic Ca	—	—
A549	Lung Adeno Ca	—	—
CHAGO	Large Cell Lung Ca	—	—
SKMES	Squamous Cell Lung Ca	—	—
PC-1	Lung Ca	—	—
PC-9	Lung Ca	—	—
PC-14	Lung Ca	—	—
T-24	Lung Ca	—	—
MLD	Normal Fibroblasts	—	—
BG-9	Normal Fibroblasts	—	—
GM2504	Normal Fibroblasts	—	—
FL	Human Amnion	—	—

The indirect immunoperoxidase staining of formalin fixed LNCaP cells by supernatants from either of the hybridoma cultures was positive in dilutions ranging from 1:200 to 1:800 while ascitic fluids harvested from mice stained LNCaP smears at dilutions from 1:50,000 to 1:400,000. The localization of immunoperoxidase staining of LNCaP cells differed from MoAb 7E11-C5 and MoAb 9H10-A4. MoAb 7E11-C5 staining was apparent over the cytoplasmic region with intensity slightly increasing toward the cell periphery (FIG. 2) MoAb 9H10-A4 produced continuous, narrow band of strong staining associated with the cell periphery (FIG. 3). The staining pattern of LNCaP cells from culture, as well as cells taken directly from nude mouse tumors was constant for each MoAb.

Antigen reactive with MoAb 7E11-C5 was best preserved by neutral formalin or cold acetone fixation. Methanol, ethanol, propanol and chloroform reduced reactivity: ethyl ether, Nonidet (0.1%) and Triton X-100 were without effect. Drying and storage at −80° for more than 3 days, regardless of fixation, as well as treatment with periodate or Bouin's fluid, destroyed the 7E11-C5 antigen, suggesting its sensitivity to oxidation. Specimens embedded in paraffin after formalin fixation retained only small fraction of the original reactivity. Antigen detected by MoAb 9H10-A4 appears to be more stable and was fully reactive after exposure to all tested fixatives, as well as after prolonged storage.

ELISA inhibitory activity was not due to the presence in tested sera of a human antibody with specificity similar to MoAb 7E11-C5, which could competitively block available antigenic sites on the LNCaP detector cells, nor were enzymatic activities of serum affecting the antigenic sites of LNCaP cells. This was shown by preincubation (up to 72 hrs.) of wells containing LNCaP cells with either "inhibitory" serum, non-inhibitory serum or PBS. The serum was then removed and MoAb 7E11-C5 activity was tested by standard ELISA procedure. No reduction in reaction intensity was observed between control wells and wells pre-incubated with inhibitory sera.

In addition, either the presence in sera of anti-murine IgG capable of binding MoAb 7E11-C5 or the existence of an unusual proteolytic activity directed against monoclonal antibodies in general, was excluded by pre-incubation of inhibitory sera with murine MoAb 9H10-A4 and showing that immunologic reactivity with LNCaP cells and membranes was unaffected.

Next, the possibility was investigated that "inhibitors" in positive CaP sera were unspecific and interacted only with the Fc portion of MoAb7E11. To this end, the inhibition of immunoreactivity of 7E11 $F(ab')_2$ antibody fragments by CaP sera was tested. The $F(ab')_2$ antibody fragments were as susceptible to inhibition by positive human sera from CaP as were the complete MoAb 7E11-C5.

Taken together, the above experiments indicate that observed ELISA inhibition results from specific immunological reaction between MoAb 7E11and corresponding antigen present in serum from some CaP patients.

The assay methodology for testing human sera from normal blood donors, non-prostatic malignancies and patients with prostatic cancer for specific binding of MoAb 7E11-C5 in limiting concentrations was established as follows:

Aliquots (125 μl) of serum were incubated (3 hrs., room temp.) with:

a) 125 μl of diluent (PBS with 0.3% bovine serum albumin, pH 7.2, sodium azide 0.05%)

b) 125 μl of MoAb 7E11(60 ng/ml in diluent)

c) 125 μl of MoAb 9H10 (6 ng/ml in diluent).

As references of total MoAb activity in the absence of serum, MoAb 7E11-C5 (30 ng/ml) and MoAb 9H10-A4 (3 ng/ml) in diluent only were used. In addition, each microtiter plate contained a set (12 wells) of external controls consisting of normal female serum preincubated separately with each MoAb and diluent.

The reaction mixtures were then incubated in a single 96 well microtiter plate (Falcon) overnight (18 hrs, 4° C.; quadruplicate wells, 50 μl/well) with air dried LNCaP cells (4×10^4 cells/well, 2.0% formaldehyde fixed for 30 min) to determine reactivity by ELISA. The results of the ELISA test (O.D. read at 490 nm) are expressed as the Specific Reactivity with MoAb 7E11-C5 factor (SR7E11 factor). The SR7E11 factor is calculated according to the formula:

$$SR_{7E11} = \frac{\text{O.D. (7E11 + diluent)}}{\text{O.D. (7E11 + serum)}} \quad \frac{\text{O.D. (9H10 + serum)}}{\text{O.D. (9H10 + diluent)}}$$

The inclusion of MoAb 9H10 in the test allows to compensate for potential differences in kinetics of binding of MoAb to target LNCaP cells in high (50%) serum concentration, as well as for unexpected presence in individual sera of interfering macromolecules (anti-murine IgG, enzymes, etc.). The MoAb 9H10-A4 strongly binds to LNCaP plasma membranes, but is unrelated in specificity to MoAb 7E11-C5 and does not react with other human cell lines, or frozen sections of normal human organs or malignant tumors. Neither normal nor CaP sera inhibit specifically MoAb 9H10-A4.

To examine the kinetics of SR7E11 factor changes, normal control and positive CaP sera were preincubated with MoAbs 7E11-C5 and 9H10-A4 for periods ranging from 3 hrs. up to 10 days. The antigen-antibody reaction was thus allowed to proceed to or near completion at limiting antibody concentrations. Table 3 shows that the SR7E11 factor of positive CaP sera significantly increases during prolonged serum-MoAb incubation, while SR_{7E11} of control sera remains low or even decreases. This further supports the notion that SR_{7E11} factor reflects the amount of antigen in serum binding MoAb 7E11. Another explanation is also plausible: sera from prostatic cancer patients could contain antiidiotypic antibodies (anti-Id) of the Ab-2 variety. Such antibodies could carry specificity and reactivity similar to the epitope associated with insoluble membranes of LNCaP cells, i.e., the epitope against which the murine monoclonal 7E11-C5 is directed. Therefore, the anti-Id could bind to 7E11-C5 and be the cause of positive results in the competitive inhibition ELISA in CaP serum.

TABLE 3

CHANGES IN SR_{7E11} FACTOR AS A FUNCTION OF PRE-INCUBATION TIME OF MoAbs WITH HUMAN SERA

	Time of Pre-incubation with MoAbs			
Serum Source	3 Hrs.	3 Days	5 Days	10 Days
Normal Female	1.28	1.33	0.98	1.19
Normal Male	1.14	1.32	1.21	1.09
Pool of Young Males	1.19	1.43	1.28	0.99
Prostatic Ca N° 1	2.07	4.11	6.83	7.34
Prostatic Ca N° 2	3.07	10.64	14.30	15.95

To establish the average numerical value of SR_{7E11} factor for normal, healthy individuals, 30 sera from RPMI Blood Bank donors were tested. The mean SR_{7E11} of this group was 1.13 ± 0.23 ($\bar{x}\pm$S.D.). No significant differences between the mean values of the SR_{7E11} factor for groups of males and females were found. For the threshold defining positive results (at the $p<0.01$ level), $x^- + 3$ S.D. was calculated to be 1.82. The value above 1.82 for SR_{7E11} was used for the classification of Specific Reactivity as positive.

Subsequently, additional 116 sera were tested: 43 from CaP patients, 7 from individuals with benign prostatic hypertrophy and 66 sera from non-prostatic malignancies. Tables, 4, 5 and 6 show the results. A strong statistical correlation emerged between the assay positive outcome and diagnosis of prostatic cancer (Table 4). In addition, as shown in Table 5, the patients with positive SR_{7E11} were more likely to be in progression than those who were negative. Similarly, a higher percentage of positive tests were among patients with widely disseminated disease vs. less advanced clinical stages. Among 66 sera from individuals with tumors of non-prostatic origin, only 3 (4.6%) tested positive (Table 6). Two of the positive sera were from females with disseminated uterine and renal carcinomas respectively. The third positive serum was obtained from young male with testicular embryonal carcinoma.

ascertain its reproducibility and specificity on a larger size sample of fresh biopsy specimen.

Cell lines 7E11-C5 and 9H10-A4 as described herein have been deposited with the American Type Culture collection, Rockville, Md., and have been assigned ATCC designation HB 10494 and ATCC Safe Deposit Designation S.D. No. 1308, respectively. The invention described and claimed herein is not to be limited in scope by the cell line deposited, since the deposited embodiment is intended as a single illustration of one aspect of the invention and any equivalent cell lines which produce a functionally equivalent monoclonal antibody are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

What is claimed is:

1. A monoclonal antibody produced by hybridoma cell line 7E11-C5, ATCC Designation HB 10494, which monoclonal antibody binds specifically to an epitope present on a membrane associated antigen of human prostatic cancer epithelium and normal prostatic epithelium and which does not bind to non-prostatic antigens present in other tissues.

2. Hybridoma cell line 7E11-C5, ATCC Designation HB 10494.

* * * * *

Exhibit 4

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D. C. 20231

75M7/0606

PENNIE & EDMONDS
1155 AVENUE OF THE AMERICAS
NEW YORK, NY 10036
ATTN: S. LESLIE MISROCK, ESQ.

MAINTENANCE FEE STATEMENT

The data shown below is from the records of the Patent and Trademark Office. If the maintenance fees and any necessary surcharges have been timely paid for the patents listed below, the notation "PAID" will appear in column 10, "status" below.

If a maintenance fee payment is defective, the reason is indicated by code in column 10, "status" below. An explanation of the codes appears on the reverse of the Maintenance Fee Statement. TIMELY CORRECTION IS REQUIRED IN ORDER TO AVOID EXPIRATION OF THE PATENT. NOTE 37 CFR 1.377. THE PAYMENT(S) WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION. IF PAYMENT OR CORRECTION IS SUBMITTED DURING THE GRACE PERIOD, A SURCHARGE IS ALSO REQUIRED. NOTE 37 CFR 1.20(k) and (l).

If the statement of small entity status is defective the reason is indicated below in column 10 for the related patent number. THE STATEMENT OF SMALL ENTITY STATUS WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION.

ITM NBR	PATENT NUMBER	FEE CDE	FEE AMOUNT	SUR CHARGE	SERIAL NUMBER	PATENT DATE	FILE DATE	PAY YR	SML ENT	STAT
1	5,162,504	283	495	----	07/202,869	11/10/92	06/06/88	04	YES	

E186 The small entity statement was not verified by an oath or a declaration.

E185 The requirements for timing as a nonprofit organization

MAINTENANCE FEE STATEMENT
STATUS CODES AND DEFINITIONS

CODE	DEFINITION
	IN REGARD TO THE MAINTENANCE FEE PAYMENT(S)
F160	The maintenance fee has already been paid. A refund of the payment has been scheduled to be sent to the fee address of record.
F161	The maintenance fee payment will not be accepted because it has been tendered too early. See 37 CFR 1.362. A refund of the payment has been scheduled.
F162	The maintenance fee payment does not properly identify the patent for which payment is to be made in accordance with 37 CFR 1.366(c). Either the U. S. application serial number or the patent number has been omitted. Both numbers are necessary to ensure proper crediting of the maintenance fee to the desired patent.
F163	The maintenance fee payment based upon certificate of mailing procedures is untimely, since it is not in compliance with the requirements of 37 CFR 1.8.
F164	The maintenance fee payment based upon "Express Mail" procedures is untimely since it is not in compliance with the requirements of 37 CFR 1.10.
F165	The maintenance fee and surcharge payment are not accepted because they have been submitted with the payment of fees for other purposes. See 37 CFR 1.366(e). A refund of the payment has been scheduled.
F166	The maintenance fee payment is not accepted because it is not immediately negotiable in the United States for the full payment of the required fee. Payment should be made in U. S. specie, Treasury notes, national bank notes, post office money orders or by certified check. See 37 CFR 1.23. The payment is returned herewith.
F167	The check or deposit account authorization is not accepted because it is unsigned. It is returned herewith.
F168	The payment received or the balance in the deposit account authorized for payment is insufficient to cover payment of the maintenance fee and surcharge, if any. Any payments accepted have been applied in accordance with the provisions of 37 CFR 1.366(e).
F169	The payment is in excess of the amount required. A refund has been scheduled.
	IN REGARD TO THE STATEMENT OF SMALL ENTITY STATUS
E180	A signature to the small entity statement is omitted.
E181	A small entity statement from each joint inventor has not been received.
E182	A small entity statement from the assignee or licensee has not been received.
E183	The requirements for filing as an independent inventor have not been met. See 37 CFR 1.9(c).
E 184	The requirements for filing as a small business concern have not been met. See 37 CFR 1.9(d).

Exhibit 5
108377

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

OCT 14 1989

Food and Drug Administration
Bethesda MD 20892

Melvin P. Fisher, Ph.D.
Director, Regulatory Sciences
Cytogen Corporation
600 College Road East
Princeton Forrestal Center
Princeton, NJ 08540

Dear Dr. Fisher:

The Center for Biologics Evaluation and Research has received your Investigational New Drug Application (IND). The following product name and BB-IND number have been assigned to this application. They serve only to identify it and do not imply that this Center either endorses or does not endorse your application.

BB-IND #: 3311

SPONSOR: Cytogen Corporation

PRODUCT NAME: Indium-InIII-Conjugated (GYK-DTPA) Murine Monoclonal Antibody (CYT-356) to Human Prostate Adenocarcinoma Antigen (LNCat)

DATE OF SUBMISSION: September 26, 1989

DATE OF RECEIPT : September 26, 1989

This BB-IND number should be used to identify all future correspondence and submissions, as well as telephone inquiries concerning this IND. Please provide an original and two copies of every submission to this file. Please include three originals of all illustrations which do not reproduce well.

It is understood that studies in humans will not be initiated until 30 days after the date of receipt shown above, unless a waiver has been requested and granted. If this office notifies you, verbally or in writing, of serious deficiencies that require correction before human studies can begin, it is understood that you will continue to withhold such studies until you are notified that the material you have submitted to correct the deficiencies is satisfactory. If such a clinical hold is placed on this file you will be notified in writing of the reasons for placing the IND on hold.

You are responsible for compliance with applicable portions of the Public Health Service Act, the Federal Food, Drug, and Cosmetic Act, and the Code of Federal Regulations (CFR). Copies of the pertinent regulations are available from this Center upon request. The following points regarding obligations of an IND sponsor are included for your information only, and are not intended to be comprehensive.

Progress reports are required at intervals not exceeding one year and are due within 60 days of the anniversary of the date that the IND went into effect (see enclosure). Any unexpected immediately life-threatening reaction which is associated with use of this drug must be reported to this Center within three working days, and all serious, unexpected adverse experiences must be reported, in writing, to this Center and to all study centers within ten working days.

Charging for an investigational drug in a clinical trial under an IND is not permitted without the prior written approval of the FDA.

Prior to use of each new lot of investigational drug in clinical trials, please submit the lot number, the results of all tests performed on the lot, and the specifications when established (i.e., the range of acceptable results).

A copy of the consent form should be submitted for each clinical study. Please provide documentation of the institutional review board approval(s) for each clinical study.

All laboratory or animal studies intended to support the safety of this product should be conducted in compliance with the regulations for "Good Laboratory Practice for Nonclinical Laboratory Studies" (21 CFR 58, copies available upon request). If such studies have not been conducted in compliance with these regulations, please provide a statement describing in detail all differences between the practices used and those required in the regulations.

Item 7a of Form FDA 1571 requests that either an "environmental assessment", or a "claim for categorical exclusion" from the requirements for environmental assessment, be included in the IND. If you did not include a response to this item with your application, please submit one. See the enclosed information sheet for additional information on how these requirements may be addressed.

Sponsors of INDs for products used to treat life-threatening or severely debilitating diseases are encouraged to consider the recently published interim rule outlined in 21 CFR 312.80 through 312.88. A copy of the new interim rule is enclosed.

Telephone inquiries concerning this IND or the use of 21 CFR 312.80 through 312.88 should be made directly to the Division of Biological Investigational New Drugs at 301/443-4864. Correspondence regarding this file should be addressed as follows:

(If U.S. Postal Services Mail):

Paul D. Parkman, M.D.
Director
Center for Biologics Evaluation and Research
Division of Biological Investigational New Drugs
ATTN: HFB 230, Building 29
8800 Rockville Pike
Bethesda, MD 20892

(If other than U.S. Postal Services Mail):

Paul D. Parkman, M.D.
Director
Center for Biologics Evaluation and Research
ATTN: Division of Biological Investigational New Drugs
HFB 230
Park Building - Room 1-29
12420 Parklawn Drive
Rockville, MD 20852

Should we have any comments following our review of this submission, we shall contact you.

Sincerely yours,

Janet Woodcock, M.D.
Director
Division of Biological
Investigational New Drugs
Center for Biologics
Evaluation and Research

Enclosures (5)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

For: U. S. Patent No. 5,162,504

Issued: November 10, 1992

Inventor: Julius S. Horoszewicz

Title: MONOCLONAL ANTIBODIES TO A NEW ANTIGENIC MARKER IN EPITHELIAL PROSTATIC CELLS AND SERUM OF PROSTATIC CANCER PATIENTS

February 5, 1997

HAND DELIVERY

Petitions Office
Special Programs
Crystal Plaza 1
Suite 520

ATTN: KAREN TYSON

In accordance with your instructions during our telephone conference on January 28, I am having hand delivered duplicate originals of an Application for Extension of Patent Term Under 35 U.S.C. 156, together with the appropriate fee. Please note that this application was originally timely filed on December 20, 1996 (see Certificate of Mailing), but was routed to the wrong office, then returned to me. I understand from our conversation that this Application will still retain the December 20, 1996 filing date.

Thank you for your assistance.

W. Scott McNees

W. Scott McNees
Reg. No. 33,964